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THE ROLE OF ADRENERGIC RECEPTORS IN NOREPINEPHRINE
STIMULATED $\dot{V}O_2$ IN MUSCLE.

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Running title:

α and β receptors in NE stimulated $\dot{V}O_2$ in muscle.

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ABSTRACT

The purpose of this study was to characterize the norepinephrine (NE) calorigenic response of an isolated perfused rat muscle preparation into α and/or β adrenergic components. The α agonist, phenylephrine, was as effective as NE in eliciting an increase in oxygen consumption by the muscle. Isoproterenol had no effect on the $\dot{V}O_2$ of the preparation. Phentolamine (.40 nM/g•min) an α blocker completely blocked the NE stimulated $\dot{V}O_2$. Propranol (.40 nM/g•min) in a sufficient dose, was also found to block the NE induced $\dot{V}O_2$ but this drug was found to be less potent. It was concluded that the NE induced calorigenic response of muscle cannot be classified either strictly as an α or β response, but unlike brown adipose tissue, the α response seems to predominate in skeletal muscle.

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1. INTRODUCTION

Since nonshivering thermogenesis is thought to be mediated by norepinephrine (NE) released from the sympathetic nerve endings, stimulation of the adrenergic receptors (either α or β) is probably a first step in evoking the calorigenic response. The adrenergic receptors are classified as α or β according to their affinity for sympathomimetic amines (Innes and Nickerson, 1970), in producing a response. Some adrenergic responses are clearly either α or β , but many others are stimulated by drugs which act on both types of receptors. The ability of NE to increase the rate of oxygen consumption is thought to be elicited through the action of the β adrenergic receptors (Schonbaum et al., 1966; Bartunkova and Jansky, 1971; Portet et al., 1971). Recent evidence has been provided suggesting that nonshivering thermogenesis in brown adipose tissue involves activation of both α and β pathways (Horwitz, 1975).

By comparing the response of various tissues to the effects of both α and β agonists and antagonists, it may be possible to determine if the calorigenic action of NE is brought about through activation of the same or different receptors in various tissues. This information may provide some insight into the mechanism of action of NE.

The purpose of the present investigation was to determine the effect of α and β agonists and antagonists on the NE evoked increase in oxygen consumption ($\dot{V}O_2$) by the perfused

rat hindlimb, and compare the responses elicited by these drugs in this skeletal muscle preparation with the responses reported for the intact animal and brown adipose tissue.

2. MATERIALS AND METHODS

Male Sprague-Dawley rats (250-300 g) were individually housed at room temperature prior to experimentation. The rats were allowed free access to food and water until surgery. Details of surgical preparation have previously been described (Grubb, 1976). After the isolated limb was cannulated the rat was killed with a pneumothorax and placed in the constant temperature chamber previously described (Grubb, 1976).

2.1. Perfusion medium

The limbs were perfused at a constant flow rate, .081 ml/min⁻¹ (in vivo range, Ross, 1966) with a semi-artificial perfusate. The perfusion medium consisted of Krebs-Ringer bicarbonate buffer, 4% bovine albumin (Sigma, fraction v), 150 mg% glucose and washed porcine erythrocytes, hematocrit 31-33%. The porcine erythrocytes were obtained at a local slaughter house and were used immediately. Details of perfusate preparation can be found elsewhere (Zivin and Snarr, 1972).

2.2. Infusion of drugs

Norepinephrine (.06 μ M/g·min levophed Bitartrate, Winthrop) was infused into the hindlimb by means of a Sage infusion pump. A small piece of tubing leading from the infusion syringe terminated in a needle. The needle was inserted into the arterial cannula. When the blockers (Propranolol-Inderal, Ayerst; Phentolamine-Regitine, CIBA) or the agonists (Phenylephrine-Neo-Synephrine, Wintrop; Isoproterenol-Isuprel, Winthrop; Epinephrine, Wintrop) were infused, a second infusion pump was employed. The doses of drugs to be infused were adjusted for leg weight, so that the doses infused per gram were identical. The doses of the infused drugs are given in the results section. All drugs were diluted with .9% NaCl to achieve the correct concentration for infusion.

To avoid infusion artifacts, saline controls (no drugs) were run in every experiment. About 10 minutes before the drug was infused, .9% NaCl was infused at the same rate as the drug was to be infused. There was usually no change in the venous oxygen tension during the NaCl infusion. Occasionally however, NaCl infusion elicited a rise in the venous oxygen tension (a decrease in metabolic rate). These few rats were eliminated from the study. When the drug infusion was started, the NaCl infusion was terminated.

2.3. Measurement of oxygen consumption

The oxygen tension of the venous perfusate was determined with an oxygen electrode as previously described (Grubb and Folk, 1976). The method of calculating the rate of oxygen consumption by the limb has been described (Grubb and Folk, 1976). All data was analyzed by the students t-test. Results are shown as mean \pm standard error, with the number of perfused limbs indicated in parenthesis.

3. RESULTS

Infusion of NE (.06 $\mu\text{M}/\text{g}\cdot\text{min}$) significantly increased the oxygen consumption by the perfused limb ($t = 5.44$, $P < .001$) (fig. 1). The metabolic response occurred almost immediately upon infusion and abated as soon as infusion of the catecolamine was terminated. Epinephrine (.06 $\mu\text{M}/\text{g}\cdot\text{min}$) also resulted in a significantly increased rate of oxygen consumption ($t = 2.91$, $P < .025$). The increased $\dot{\text{V}}\text{O}_2$ resulting from the epinephrine infusion was not significantly different from the $\dot{\text{V}}\text{O}_2$ elicited by NE infusion ($t = .78$, n.s.) (fig. 1).

3.1. Adrenergic agonists

Infusion of the β agonist, isoproterenol, failed to elicit an increase in oxygen consumption at any of the three

doses employed (fig. 2).

Infusion of the α agonist, phenylephrine ($.06 \text{ mM/g} \cdot \text{min}$) resulted in an increase in oxygen consumption of the same magnitude as that elicited by NE (fig. 2). There was no significant difference in the increased $\dot{V}\text{O}_2$ elicited by phenylephrine as compared to NE ($t = 1.35$, n.s.).

3.2. Adrenergic antagonists

The action of the adrenergic antagonists did not depend on the time of infusion relative to the time NE was infused. Infusion of the blocker 5-10 minutes prior to NE infusion was equally as effective as infusion of the blocker during the NE infusion (table 1). Therefore the protocol followed in most of the blocker experiments was to first infuse NE (usually for 2-3 minutes) and then start the infusion of the blocker. An immediate decrease in NE stimulated $\dot{V}\text{O}_2$ was seen upon infusion of the blocker.

Infusion of the β blocker propranolol ($4.0 \text{ } \mu\text{M/g} \cdot \text{min}$) resulted in a decrease in the $\dot{V}\text{O}_2$ elicited by NE (fig. 3), although this degree of blocking was not significant ($t = 1.34$, n.s.). Infusion of a higher dose of propranolol ($40 \text{ } \mu\text{M/g} \cdot \text{min}$) was effective in completely blocking the increased metabolic rate ($t = 3.24$, $P < .025$). The $\dot{V}\text{O}_2$ following infusion of the high dose of propranolol was slightly lower than the basal level of oxygen consumption (fig. 3).

The duration of the blocking action of propranol was relatively short in that an increase in oxygen consumption could be elicited by infusing NE after infusion of the blocker had been terminated 10 minutes previously.

The response of the muscle preparation to the α blocker, phentolamine, was both qualitatively and quantitatively different from that evoked by propranol. Infusion of phentolamine (.4 ~M/g·min) during the NE infusion, completely blocked the NE stimulated $\dot{V}O_2$, and the metabolic rate in the presence of phentolamine was not significantly different from the control values ($t = .073$, n.s.), (fig. 4).

The duration of the blocking action of phentolamine was much longer than that of propranol. It was not possible to elicit the NE induced increase in $\dot{V}O_2$ even after 60 minutes had elapsed after infusion of the blocker was terminated.

4. DISCUSSION While the exact mechanism by which NE increases the $\dot{V}O_2$ of tissue is not known, in brown fat NE appears to modify several properties of the cell membrane, which may be related to the calorigenic effect of the catecholamine. NE has been shown to (1) depolarize the brown fat cell membrane (Horwitz and Smith, 1972), (2) decrease the resistance of the cell membrane, allowing an increased membrane permeability (Horowitz, 1971), and (3) increase the activity of the ouabain-sensitive Na^+/K^+ ATPase (Horwitz, 1973). In brown fat the adrenergic agonists and antagonist have been shown to mimic or inhibit respectively these membrane changes induced by NE. Much less work has been done on skeletal muscle, but it has been reported that NE

causes a depolarization of skeletal muscle (Teskey and Horowitz, 1975).

Since no studies could be located in which the effect of α , β agonists and antagonist on skeletal muscle $\dot{V}O_2$ was investigated, the results reported in this paper will be compared to the effects of these drugs on the intact animal and isolated brown adipose tissue.

The response of the perfused limb to phenylephrine was studied as the drug has been found to have almost exclusively α activity (Innes and Nickerson, 1970). In the present investigation it was found that phenylephrine elicited an increase in oxygen consumption of the same magnitude as that produced by NE. Horwitz (1975) found that infusion of phenylephrine to cold acclimated rats, was followed by membrane depolarization of brown fat, an increase in Na^+/K^+ ATPase activity, and an increase in oxygen consumption by intrascapular brown fat cells.

Brown fat from cold acclimated rats has been shown to respond to the β agonist, isoproterenol, by increasing its rate of oxygen consumption (Horwitz, 1975). In the present investigation there

was no change in the $\dot{V}O_2$ of skeletal muscle upon infusion of isoproterenol in doses up to 6.0 M/g·min. A dose of isoproterenol 100 times greater than the effective dose of phenylephrine failed to increase the $\dot{V}O_2$ of the hindlimb.

Thus in skeletal muscle, only the α agonist mimicked the metabolic action of NE, whereas in brown adipose tissue both the α and β agonist were effective in increasing the rate of oxygen consumption (Horwitz, 1975), although the β agonist seems more effective in this regard (Horwitz, personal communication). It should be noted that the brown fat was from cold acclimated animals whereas limbs of the animals employed in the present investigation were from ~~warm-acclimated animals~~ ^{animals acclimated & room temperature}.

Phentolamine, an α blocker, completely blocked the NE stimulated $\dot{V}O_2$. The duration of the blocking effect was long. An increase in $\dot{V}O_2$ above the basal level could not be elicited by NE even after infusion of phentolamine had been terminated 1 hour previously. In brown fat, phentolamine is reported to block the membrane depolarization (Horwitz et al., 1969), but not the calorogenic effect of sympathetic nerve stimulation (Horwitz et al., 1969; Eskine, 1975). Thus, it appears that adrenergic α blockade has qualitatively different responses in the two types of tissue. However, it may be that this blocking agent is more effective in inhibiting the response brought about by circulating catecholamines (the perfused limb) than it is in blocking the response elicited by NE released at nerve endings (brown fat).

The β blocker, propranol, was effective in blocking the NE stimulated $\dot{V}O_2$ in the perfused muscle. However, a dose of propranol 100 times greater than phentolamine was needed to completely block the catecholamine stimulated metabolic rate. When propranol is given to the intact animal it has been found effective in blocking the NE mediated calorigenic response (Portet *et al.*, 1971; Schonbaum *et al.*, 1966; Hissa and Hirsimaki, 1971). In brown fat propranol was found effective in blocking the calorigeneous (Erskine, 1975) and stimulation of Na^+/K^+ ATPase activity (Horwitz and Smith, 1972) evoked by adrenergic nerve stimulation. The blockage of the increased Na^+/K^+ ATPase was found to be 100 times more sensitive to propranol than to phentolamine (Horwitz and Smith, 1972).

It can be concluded that the NE stimulated $\dot{V}O_2$ in skeletal muscle cannot be classified either strictly as an α or β response. While only an α agonist was capable of mimicking the NE response, both the α and β antagonists were capable of blocking the NE stimulated $\dot{V}O_2$, although the α agonist was more potent. Skeletal muscle appears to differ from brown adipose tissue in that the β agonist was ineffective in increasing the $\dot{V}O_2$ in muscle, whereas the β agonist was more effective than the α agonist in increasing the metabolic rate of the adipose tissue (Horowitz *Personal communication*). The response of the two tissues to phentolamine also differs. This drug was very effective in completely blocking the NE stimulated $\dot{V}O_2$ in the hindlimb, but in brown fat this α antagonist is reported to have no effect on the calorigenic response of the tissue (Horwitz *et al.*, 1969). This may

indicate that different mechanisms of action are involved
in the norepinephrine stimulated $\dot{V}O_2$ in the two tissue types.

Acknowledgements.

This study was supported by The Arctic Institute of North America with approval and financial support of the Office of Naval Research under contract number N00014-75-C-0635 (subcontract ONR-455) and Postdoctoral Fellowship 1 F 32HL-05108-01. All illustrations were kindly provided by Mary A. Folk.

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TABLE 1

Per cent change in $\dot{V}O_2$ from NE stimulated level.

Dose	<u>Propranolol infused</u>	
	Before NE	After NE
4.0 nM/g·min	↓ 21%	↓ 20%
40 nM/g·min	↓ 116%	↓ 125%

FIGURE LEGENDS

Fig. 1. Effect of norepinephrine and epinephrine infusion on the rate of oxygen consumption by an isolated, perfused rat hindlimb (muscle preparation). The number shown in bars is the number of hindlimbs perfused. The bars are mean \pm S.E.M.

Fig. 2. The effect of β agonist, isoproterenol, and α agonist, phenylephrine, on the rate of oxygen consumption of an isolated rat hindlimb. Bars are mean \pm S.E.M.

Fig. 3. The effect of an α blocker, phentolamine, on the NE induced increase in oxygen consumption of an isolated rat hindlimb. Bars are mean \pm S.E.M.

Fig. 4. The effect of a β blocker, propranolol, on the NE induced increase in oxygen consumption of an isolated rat hindlimb. Bars are mean \pm S.E.M.

Figure 1.

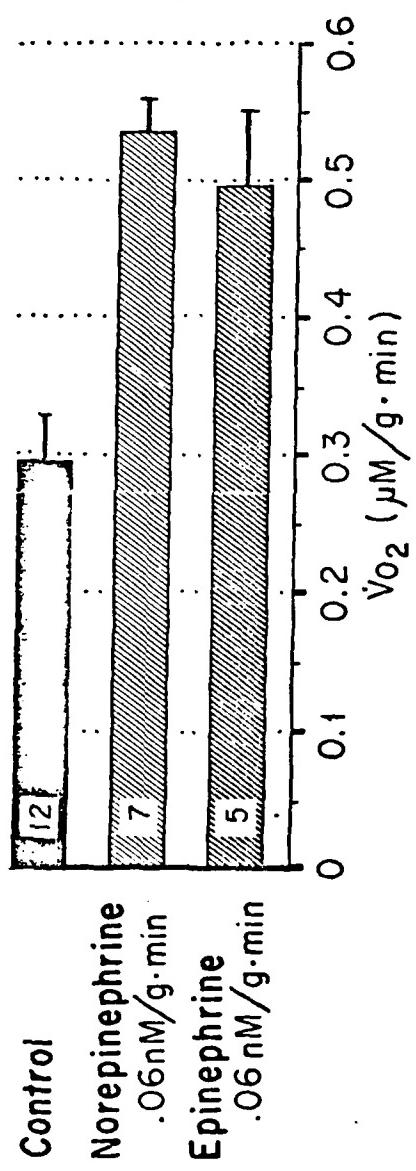


Figure 2.

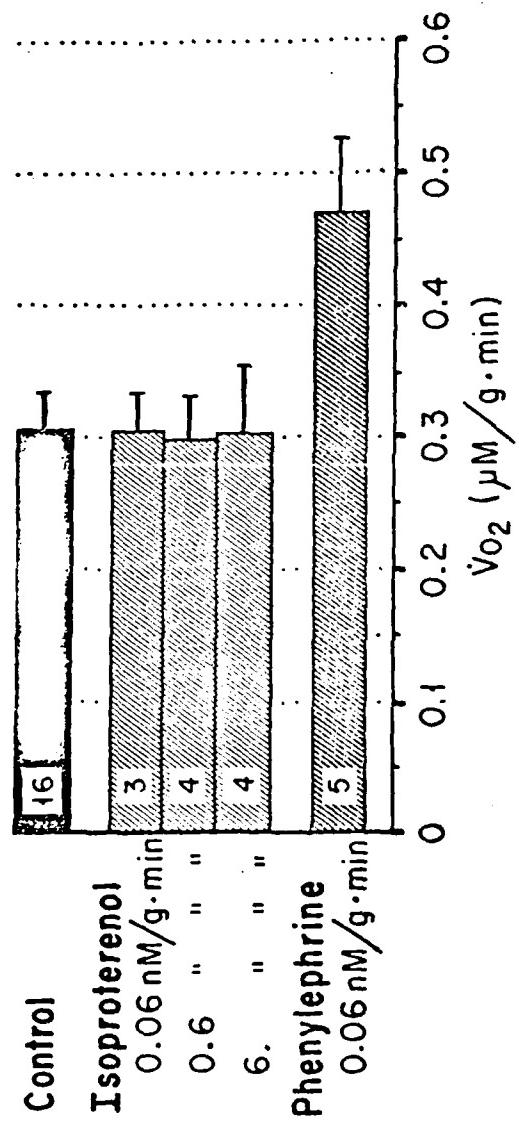


Figure 3.

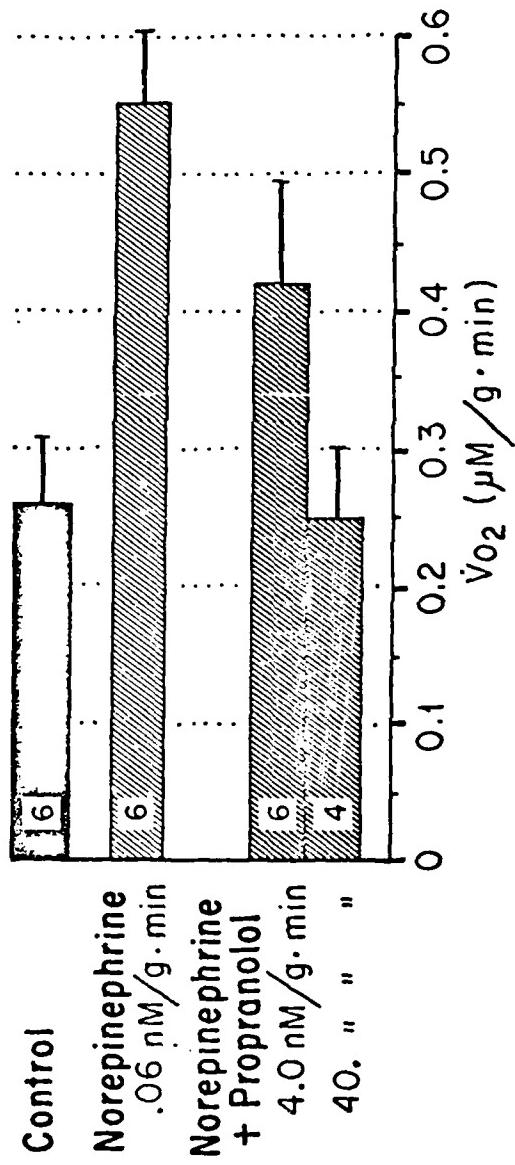
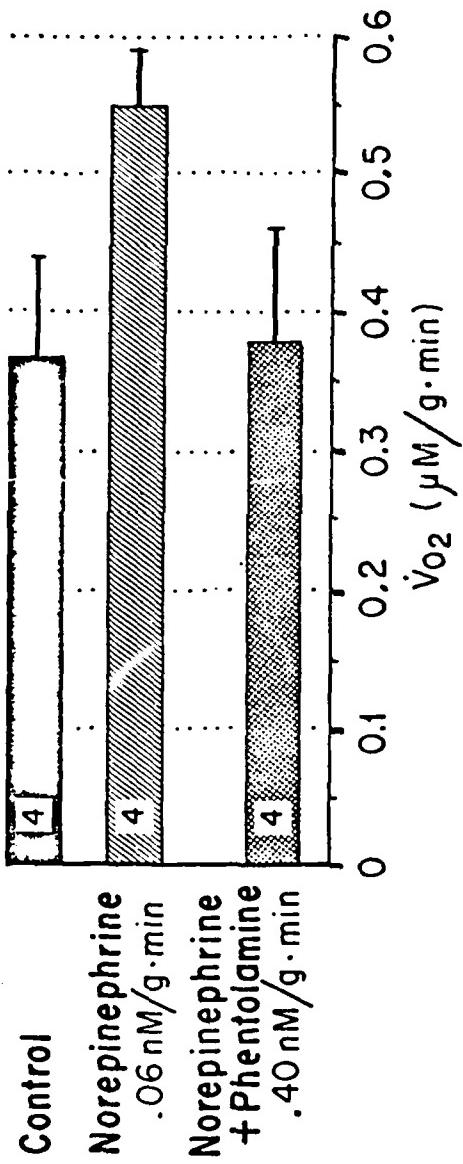


Figure 4.



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